P-gp transfer and acquired multi-drug resistance in tumors cells

Pierre Magal

Institut de Mathématiques de Bordeaux
Université Victor Segalen Bordeaux 2

ACMAC University of Crete, Grece Oct 4-6 2012
Role of P-glycoprotein (P-gp) in cancer

- P-gp is an ABC-transporter that uses ATP as an energy source to pump many cytotoxic substances out of cells.

- More than 40% of human cancers express P-glycoprotein at levels sufficient to confer Multi-Drug Resistance (MDR*)

- Expression of P-gp can be induced by treatment: leukemias, myeloma, lymphomas, breast, ovarian cancer;

- Native P-gp expression (before treatment): colon, kidney, pancreas, liver;

*Sauna, Mol. Cancer Ther. 2007
Direct intercellular transfer of P-gp

Levchenko et al. co-cultured sensitive and resistant cancer cells and used a fluorescent antibody to measure the level of P-gp expression, Proc. Nat. Acad. Sci. USA 102, 2005
Spatial organization of growing MCF-7 Human Breast cancer cells


**Figure 2** Spatial organization of MCF-7 and MCF-7/Doxo in co-cultures. To obtain phase contrast micrographs of growing MCF-7 variants in co-cultures, dishes were seeded with a 50:50 mixture of MCF-7:MCF-7/Doxo at day 0. Morphological differences permit an immediate identification of each cell subpopulation. MCF-7 appeared birefringent and round (boxes) whereas MCF-7/Doxo are more flat and spread (ellipses). Note that the cells remained organized in well-delimited islets.
Fluorescence of P-gp by flow cytometry

**Day 0**

Fluorescence on Day 0 in log scale

**Day 3**

Fluorescence on Day 3 in log scale

**Day 4**

Fluorescence on Day 4 in log scale

**Day 5**

Fluorescence on Day 5 in log scale

**Day 6**

Fluorescence on Day 6 in log scale
Fluorescence measuring the efflux Activity of P-gp by flow cytometry
Mass preservation

Graphs C and D illustrate the total mass of proteins and activity over time in days, respectively.
Comparison co-cultured cells at day 0 and day 6
Direct transfers


\[
\begin{aligned}
\partial_t u(t, p) &= 2\tau [T(u(t,.))(p) - u(t, p)], \text{ for } t \geq 0 \\
u(0,.) &= \varphi(.) \in L^1_+(R) \\
T(\varphi)(p) &= \begin{cases} 
\frac{(\varphi \ast_{f} \varphi)(p)}{\int_{R} \varphi(s)ds} & \text{if } \int_{R} \varphi(s)ds > 0 \\
0 & \text{otherwise}
\end{cases}
\end{aligned}
\]

\[
(\varphi \ast_{f} \varphi)(p) = \int_{R} \varphi(p + f(l)l)\varphi(p - f(l)l)dl
\]

whenever \(f(l)\) is constant we obtain a 1-dimensional Boltzmann like equation
Consider a transfer event in which one partner in the pair has value \( \hat{p} \) before the event and the other partner in the pair has value \( p \) after the event. The 2 possibilities are

\[
\begin{align*}
p & \rightarrow \hat{p} \quad \hat{p} = p - f(p - q) \\
q & \rightarrow \hat{q} \quad \hat{q} = q + f(p - q)
\end{align*}
\]
System restricted to $(0,1)$

\[
\begin{align*}
\partial_t u(t, p) &= 2\tau [T(u(t,.))(p) - u(t, p)], \text{ for } t \geq 0 \text{ and } p \in (0,1) \\
u(0,.) &= \varphi(.) \in L^1_+(0,1)
\end{align*}
\]

\[
T(\varphi)(p) = \begin{cases} 
\left( \varphi \ast f \varphi \right)(p) & \text{if } \int_{\mathbb{R}} \varphi(s)ds > 0 \\
\int_{\mathbb{R}} \varphi(s)ds & \text{otherwise}
\end{cases}
\]

\[
\left( \varphi \ast_{f} \varphi \right)(p) = \int_{\mathbb{R}} \varphi(p + f(l)l)\varphi(p - f(l)l)dl
\]

where

\[
\varphi(x) = \begin{cases} 
\varphi(x), & \text{if } x \in (0,1) \\
0, & \text{otherwise.}
\end{cases}
\]
Theorem.

1. For each initial datum $\phi_0$ in $L^1_+(0, 1)$, the transfer model has a global positive solution. Moreover, for all $t \geq 0$ and $n = 0, 1$, $E_n(u(t)) = E_n(\phi_0)$ (thus, cell count and cell p-mass are conserved).

2. For each initial datum $u_0$ in $L^1_+(0, 1)\{0\}$ there exists a Radon measure $\omega$ on $[0,1]$ such that for every $\phi$ in $C[0,1]$ the solution of the transfer model satisfies

$$\lim_{t \to \infty} \langle u(t), \phi \rangle = \langle \omega, \phi \rangle$$

3. If $f$ is constant, then for each initial datum $u_0$ in $L^1_+(0, 1)\{0\}$ the solution of the transfer model converges to a dirac measure in the weak* topology of $C[0,1]^*$ with dirac mass at $E_1(u_0)/E_0(u_0)$. 
Parameters estimations for the transfer model

Figure 6 Estimation of parameters for the transfer model. This graph corresponds to the distribution of P-gp activity at day 6 of co-culture. The initial distribution of P-gp activity at day 0 (not shown here) was obtained from a mixture of 50:50 MCF-7/MCF-7/ Doxo cells analyzed by cytometry as in Figure 4. The transfer model was run over 6 days. The dotted curve corresponds to the efflux activity of the co-culture measured at day 6, and the solid curve corresponds to the distribution of activity derived from model. The fitting parameters are given above the curves and were obtained by least squares minimization.
Model with two fractions
PM and P. Zongo, In preparation

Data

One fraction transferred

Two fraction transferred
Direct immunodetection of P-gp transfer in cocultures. Sensitive MCF-7 were tagged with the long-lasting fluorescent probe CellTracker green prior to coculture. A mixture of 50:50 MCF-7:MCF-7/doxo was cocultured during 0 to 7 days (D0-D7) on glass coverslips. P-gp was immunodetected with a phycoerythrin-conjugated UIC-2 monoclonal antibody (red fluorescence) by confocal laser scanning microscopy on non-dispersed (left column) or after mechanical dispersion of the cells (right column). Note that the green fluorescence progressively fades by dilution within daughter cells during cell division. From D3 (arrow heads) to D7, sensitive green MCF-7 show an increasing red P-gp-specific membrane staining, restricted at the plasma membrane.
XIII. Cells are interconnected by Tunneling nanoTubes (TnTs)
Indirect transfers

J. Dyson, F. Le Foll, and PM, in preparation

Adsorption of MDR+ microparticles on MCF-7
Schematic representation of indirect transfers

Cells ↔ Liquid

P-gp
A model for indirect transfers

\[
\begin{cases}
\partial_t n(t,r) = \partial_p \left[ \beta \eta(r)n(t,r) \right] - \partial_p \left[ \alpha p(t) \eta(r)n(t,r) \right], r \in I \\
\frac{d}{dt} p(t) = \left[ \beta \int_I \eta(r)n(t,r)dr \right] - \left[ \alpha \int_I \eta(r)n(t,r)dr \right] p(t)
\end{cases}
\]

with \( n(0, \cdot) = n_0 \in L^1_+(I) \), and \( p(0) = p_0 \geq 0 \)
Abstract reformulation

\[
\begin{aligned}
\frac{dn(t)}{dt} &= (\beta - \alpha p(t))An(t), \\
\frac{dp(t)}{dt} &= x^*(n(t))(\beta - \alpha p(t))
\end{aligned}
\]

where

\[x^*(\varphi) = \int \eta(r)\varphi(r)dr\]

and

\[A \varphi = - (\eta \varphi)'\]

\[D(A) = \{\varphi \in L^1(I) : (\eta \varphi) \in W^{1,1}(I)\}.
\]
A model with both direct and indirect transfers

Setting \( q(t) = (\beta - \alpha p(t)) \) we obtain

\[
\begin{align*}
\frac{dn(t)}{dt} &= q(t) A n(t) + 2\tau[H(n(t)) - n(t)] \\
\frac{d\alpha^*(n(t)) q(t)}{dt} &= -\alpha^*(n(t)) q(t)
\end{align*}
\]

**Theorem:** Assuming that

(i) \( A \) is the infinitesimal generator of a strongly continuous group of bounded linear operators on \( L^1(I) \);

(ii) \( H \) is positive and Lipschitz continuous.

**This problem generates a unique positive semiflow.**
A model spatial motion of cells
A. Ducrot, F. Le Foll, PM, H. Murakawa, J. Pasquier, G. F. Webb,

Basic ingredients incorporated into the model:
• Darcy's law
• Cell size
• Quiescence
• Contact inhibition
Simplified model

\[ u_t = \nabla \cdot (u \nabla u) + (bG(K_r * u) - \mu)u \]

where

\[ G(0) = 1 \text{ and } \lim_{x \to +\infty} G(x) = 0 \]

and

\[ K_r(x) = \begin{cases} 
1 & \text{if } |x| < r \\
0 & \text{otherwise}
\end{cases} \]
A model for two species

A. Ducrot PM and H. Murakawa, In preparation

\[ u_t = \nabla . (u \nabla (u + v)) + (b_u G(K_r \ast (u + v)) - \mu_u) u \]

\[ v_t = \nabla . (v \nabla (u + v)) + (b_v G(K_r \ast (u + v)) - \mu_v) v \]
Similar model with both resistant and non resistant cells
A model with non local transport term

\[
    u_t = \nabla.(u\nabla(\rho^*(u + v))) + (b_u G(K_r^*(u + v)) - \mu_u)u
\]
\[
    v_t = \nabla.(v\nabla(\rho^*(u + v))) + (b_v G(K_r^*(u + v)) - \mu_v)v
\]

where \( \rho \) is smooth, positive and even map, and satisfies

\[
    \int_{\mathbb{R}} \rho(x)dx = 1.
\]

**Advantage:**
- The existence and positivity of solutions can be proved
- This model has a nice segregation property.

**Difficulty:** We don’t really understand the diffusive properties in function of \( \rho \).
Single non-local transport equation

Consider now

$$\partial_t u = \partial_x (u \partial_x (\rho * u))$$

**Question:** When $\rho$ converges to a Dirac mass do we have a numeric convergence at least for certain solutions of

$$\partial_t u = \partial_x (u \partial_x u)$$

**Few references on nonlocal diffusion:**
T. Hillen and K. Painter (2001)
M. Burger and M. Di Francesco (2008)
Convergence to the Barenblatt solution

Rescaling the function

\[ \rho(x) = \begin{cases} \frac{1}{e^{1-x^2}} & \text{if } x^2 < 1 \\ 0 & \text{otherwise} \end{cases} \]
When the Fourier transform of $\rho(x)$ becomes positive. So for example by rescaling

$$\rho(x) = e^{-x^2}$$


